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Effects of Turbidity on Fluridone Treatments for Curlyleaf Pondweed Control

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PURPOSE: A small-scale study was conducted to evaluate fluridone (1-methyl-3-phenyl-5-[3-trifluoromethyl)phenyl]-4(1*H*)-pyridinone) efficacy under varying levels of turbidity when controlling curlyleaf pondweed (*Potamogeton crispus* L.). There is no information on the effect of turbidity on fluridone efficacy against curlyleaf pondweed. It is unknown if turbidity impacts aqueous fluridone concentrations in the water column, thereby lessening herbicide efficacy and reducing control, or whether turbid conditions create additional stress that could intensify herbicide effects, causing greater plant injury and deterioration.

BACKGROUND: Curlyleaf pondweed occurs in turbid rivers and lakes throughout the world (Kunii 1989, Nichols 1992, Coops et al. 1999). In the United States, it is an invasive aquatic macrophyte, and grows in many turbid windswept lakes in the Great Lakes region, including Lake Benton. Located in Lincoln County, Minnesota, Lake Benton (surface area=1151 ha, mean depth=2 m) is a large, relatively shallow lake surrounded by homes, agricultural fields, and pastureland (Heiskary et al. 2003). Dense stands of curlyleaf pondweed occupy most of the lake with populations concentrated in the upper and lower ends of the lake. Few other submersed aquatic plants are able to survive in turbid windswept lakes like Lake Benton (Engel and Nichols 1994, Hansel-Welch et al. 2003).

Poor water clarity caused by turbidity limits light penetration and restricts photosynthesis (Barko et al. 1986). Turbid-tolerant plants are those species that bolt to the water surface from energy stored from vegetative propagules or root stocks right after ice-out before water clarity declines with increasing turbidity (Engel and Nichols 1994). These species usually have complex and deep root systems to withstand wind-driven waves that scour the lake bottom, suspending sediments in the water column (Engel and Nichols 1994). The anatomy and life cycle of curlyleaf pondweed enables it to grow in turbid conditions. In a survey of Wisconsin lakes, Nichols (1992) found that curlyleaf pondweed was positively correlated with turbidity. Other pondweeds with tubers or turions as their primary mode of reproduction, such as sago pondweed (*Stukenia pectinata* (L.) Börner) and floating-leaf pondweed (*Potamogeton natans* L.), also survive in turbid waters (Engel and Nichols 1994). In fact, isolated clumps of sago pondweed are found in Lake Benton.

The dominance of curlyleaf pondweed in Lake Benton has interfered with recreation on the lake and contributed to blue-green algal blooms (Heiskary et al. 2003). The herbicide fluridone has been applied to alleviate these problems. Greenhouse studies conducted by the U.S. Army Engineer Research and Development Center (ERDC) Chemical Control and Physiological Processes Team have demonstrated that applying doses of 4 to 5 $\mu g\ L^{-1}$ active ingredient (ai) fluridone for an exposure time of at least 56 days controls curlyleaf pondweed by reducing growth and limiting turion production (Poovey et al. 2005, 2006).

There is no information, however, on the effect of turbidity on fluridone efficacy against curlyleaf pondweed. Turbidity in surface waters is caused by suspended organic matter, sediment, and other inorganic particles. Clays also may comprise a part of the suspended sediment found in turbid

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waters. Because these suspended substances are negatively charged, they have the potential to adsorb cationic herbicides and render them unavailable for plant uptake. For example, turbidity has interfered with efficacy of diquat in small-scale studies that tested a variety of suspended sediment types and levels (Hofstra et al. 2001, Poovey and Getsinger 2002, Poovey and Skogerboe 2004). On the other hand, because of their binding properties, clay carriers are used in herbicide pellet formulations, including some formulations of fluridone. The release rate of the fluridone from these clay carriers results in prolonged herbicide exposure periods, but also suggests that high turbidity levels may interfere with the availability, and therefore plant uptake, of fluridone.

A small-scale study was conducted to evaluate fluridone efficacy under varying levels of turbidity when controlling curlyleaf pondweed. Sediment collected from Lake Benton was used to create turbidity at levels found in the lake during early summer. The authors predicted that clays found in the Lake Benton sediment would not appreciably affect fluridone efficacy. Since curlyleaf pondweed is found in turbid field conditions, simulated turbidity in the lab was not expected to impact its growth or response to herbicide treatment.

MATERIALS AND METHODS: This study was conducted at the ERDC, Vicksburg, MS in a controlled-environmental growth chamber (58 m^2). Ambient conditions were an air temperature of 21±2°C, a mean light intensity that ranged from 254±7.43 to 277±8.61 μmol m^{-2} sec^{-1}, and a photoperiod of 14h:10h, light:dark cycle. Lighting was provided by a combination of 400-watt, high-pressure sodium and metal halide bulbs.

Curlyleaf pondweed turions were obtained from Eau Galle Reservoir, WI, and sprouted in the growth chamber under ambient conditions for 4 weeks. Mean turion fresh weight (±1 SE, n=28) was 0.18 ±0.02 g. Three sprouted turions (shoot length=1 to 5 cm) were planted in plastic containers (volume=1 L, diameter=11 cm, height=13 cm) filled with natural lake sediment that was amended with 100 mg L^{-1} ammonium chloride and 1 mg L^{-1} Osmocote® fertilizer (18-6-12) to provide adequate nutrients for plant growth. Sediment was capped with a 1-cm layer of coarse-grit sand to prevent dispersion of sediment particles in the water column. Thirty-six vertical aquaria (48-L capacity) were filled with culture solution (Smart and Barko 1985) and three planted containers were placed in each aquarium. To obtain even biomass in all replicates, plants were allowed to grow for 6 weeks before herbicide application (shoot length ~40 cm).

A field-collected sediment from Lake Benton, MN, was used to create turbid conditions in the water column. The sediment was classified as a sandy clay loam with 70 percent sand, 5 percent silt, and 25 percent clay (A&L Analytical Laboratories, Memphis, TN). An X-ray diffraction analysis (<2μm) showed that the clay component was primarily quartz [SiO_2]with calcite [$CaCO_3$] and dolomite [$CaMg(CO_3)_2$] present. Expandable clays, such as kaolinite [$Al_2Si_2O_5(OH)_4$], illite [$(K,H_3O)Al_2Si_3$ $ALO_{10}(OH)_2$], and chlorite [$(Mg,Fe,Al)_6(Si,Al)_4O_{10}(OH)_8$] were present in small amounts (ERDC Geotechnical and Structures Laboratory). Sediment pH was 7.9 and cation exchange capacity (CEC) was 25.6 meq 100 g^{-1}.

Turbidity levels in Lake Benton ranged from 19 to 27 nephlemetric turbidity units (NTU) in June 2004; therefore, low (~10 NTU) and high (~20 NTU) turbidity levels were created in the water column by dissolving 20 and 40 g of sediment, respectively, in 250 ml water to create a slurry. These slurries were stirred continuously for 18 hr. For each turbidity treatment, the slurry was

mixed with 2 L water, then poured into a treatment aquarium 24 hr before herbicide application. Some sediment particles coated plant stems and leaves. Aquaria without turbidity (none=~1 NTU) were also included as references.

Stock solutions of fluridone as Sonar® AS (SePRO Corp., Carmel, IN) were prepared based on percent active ingredient (ai; 479 mg ai L^{-1}), then applied subsurface to aquaria using a pipette to achieve target concentrations of 3, 4, and 5 µg ai L^{-1}. Herbicide exposure lasted for 56 days. Untreated references (0 µg ai L^{-1}) compared plant growth in the absence of herbicide dosage.

Water samples were collected in 30-mL, high-density polypropylene amber bottles from one replicate of each treatment at 3, 14, 35, and 56 days after treatment (DAT). Samples were refrigerated at 4°C until analysis. At the end of the study, samples were shipped to SePRO Corp where they were analyzed by enzyme-linked immunoassay (ELISA) to quantify actual concentrations and to monitor herbicide degradation.

During herbicide exposure, turbid conditions were maintained in aquaria by additions of sediment slurry and vigorous stirring with a PVC pipe (length=1 m) as necessary supplemented with continuously bubbling air into the water column. Turbidity was measured with a portable turbidimeter (Model 2100P, Hach Co.). After 21 days, suspended sediment was allowed to settle to the bottom of the aquaria until the end of the study to prevent damage to plant shoots from stirring and slurry additions.

Water column pH was measured pretreatment with a Multi-Parameter Probe (Model 556, YSI Instruments). Water temperatures were monitored continuously with an Optic Stowaway Temperature Probe (Onset Computer Corp.).

At pretreatment, 35, and 56 DAT, one planted container was removed from each aquarium. Herbicide efficacy was assessed by measuring shoot biomass and turion production. Shoot biomass was collected, dried for 48 hr at 70 °C, weighed, and reported as grams dry weight (DW). If turions were present, they were collected from each plant and counted. Turions were also collected from the bottom of each aquarium at 56 DAT.

Treatments were assigned to individual aquaria in a completely randomized manner and replicated three times. Biomass and turion data were subjected to a two-way analysis of variance (ANOVA) to determine herbicide and turbidity effects. If statistical differences occurred between treatments, means were separated using the Tukey test ($p \leq 0.05$).

RESULTS AND DISCUSSION: Initial turbidity levels fluctuated with a low level of ~10 NTU and a high level of >20 NTU (Figure 1). Turbidity was difficult to maintain because the clay component (>2µm) of the Lake Benton sediment was mostly quartz, which has a specific gravity >1, and would settle to the bottom of the aquaria a few hours after application. Expandable clays, such as kaolinite and bentonite, have specific gravities of 0.3 and 0.6, respectively, and these components remain suspended. Turbidity in aquaria without sediment additions (no treatment) increased from 0.3 at pretreatment to 4.0 NTU at 56 DAT (Figure 1), as some planktonic algae were observed in fluridone-treated aquaria.

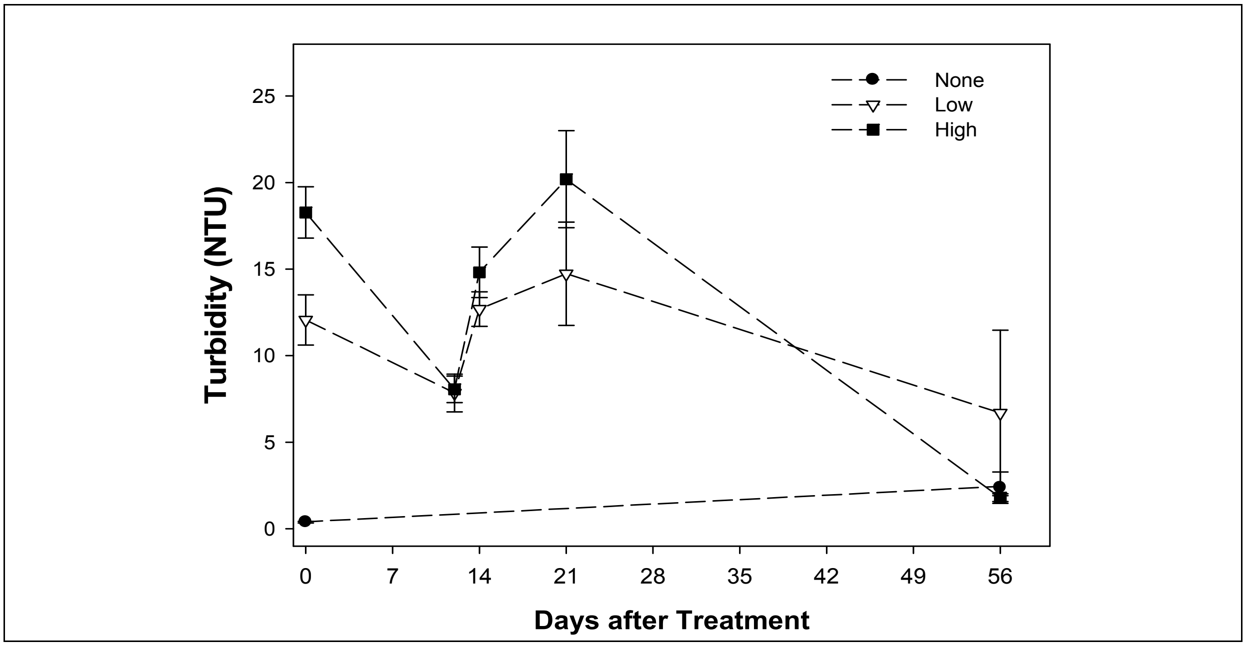

Figure 1. Turbidity created from Lake Benton sediment pretreatment, 12, 14, 21, and 56 days after fluridone application (0, 3, 4, and 5 µg ai L^{-1}). Means are ±1 SE (n=12) for all treatment aquaria, including references, for each turbidity level.

Fluridone Residues. Aqueous fluridone concentrations were not affected by turbidity created by the Lake Benton sediment. Results from the fluridone residue analyses showed that target concentrations were met 3 DAT for all treatments (Table 1). Moreover, herbicide doses that suppress curlyleaf pondweed growth and reproduction (Poovey et al. 2004, 2005) were present in all treatment aquaria through the course of the experiment. By 56 DAT, fluridone concentrations were still >2.5 µg ai L^{-1} in most treatment aquaria. Like diquat, adsorption of fluridone by suspended sediment may be dependent upon amount and type of clay present in the water column (Weber 1980, Weber et al. 1986). The clay component of the Lake Benton sediment that was used for creating turbidity was mostly quartz, with the expandable clays of kaolinite, illite, and chlorite present only in small amounts. Water column pH also affects fluridone adsorption to clays. Because fluridone is weakly basic, its adsorption to sediment particles is higher with water column pH levels <4.0 (Weber 1980, Weber et al. 1986, Yaron-Marcovich et al. 2004). Water column pH in this study was 8.5, which probably hindered fluridone adsorption to Lake Benton suspended sediment.

Plant Growth. All fluridone treatments without turbidity were effective in reducing shoot biomass of curlyleaf pondweed by 45 to 65 percent compared to the untreated reference after a 35-day exposure period (Figure 2A). After a 56-day exposure period, there were no significant differences between treatments (herbicide rate, $F=1.886$, $p=0.159$); however, shoot biomass of treated plants was reduced beyond the initial biomass measured at pretreatment levels (Figure 3A). Although plants were either deteriorating or dead, one or two green shoots were still present in many of the fluridone treatments and comprised the remaining biomass at the final harvest (Figure 4). These results support those from greenhouse studies in which fluridone rates of 4 and 5 µg ai L^{-1} decreased curlyleaf pondweed shoot biomass by at least 60 percent with exposure periods of 49 to 56 days (Poovey et al. 2005, 2006). It is likely that longer exposure periods would result in even greater

control in these treatments based on previous concentration/exposure time studies conducted with fluridone against Eurasian watermilfoil (*Myriophyllum spicatum* L.) and hydrilla (*Hydrilla verticillata* (L.f.) Royle) (Netherland et al. 1993; Netherland and Getsinger 1995a, 1995b).

Table 1. Herbicide concentrations in aquaria treated with 3, 4, and 5 µg ai L^{-1} fluridone under turbid conditions (none, low and high) at 3, 14, 35, and 56 days after treatment (DAT).

	Herbicide Concentrations µg ai L^{-1}			
Treatment	3 DAT	14 DAT	35 DAT	56 DAT
3 µg ai L^{-1}				
None	2.7	4.3	2.9	2.8
Low	2.7	3.2	2.3	2.7
High	4.4	3.4	3.2	—[1]
4 µg ai L^{-1}				
None	5.8	3.6	3.8	3.2
Low	4.3	3.8	—	3.3
High	5.0	4.5	3.6	3.6
5 µg ai L^{-1}				
None	6.2	—	3.0	2.4
Low	8.2	3.9	—	2.8
High	6.1	5.4	3.1	2.6

[1] No data.

There were no consistent turbidity effects on fluridone treatments at 35 DAT (Figure 2A). Although the interaction of fluridone concentration with turbidity was not statistically significant due to the large variances in plants treated with 3 µg ai L^{-1} ($F= 1.562$, $p=0.201$) at 56 DAT, there was a trend of shoot biomass decreases for plants treated with fluridone under turbid conditions compared to the references (Figure 3A). Compared to plants dosed with fluridone without turbidity, control in 5 µg ai L^{-1} fluridone treatments was increased by ~55 percent with a low level of turbidity and by 65 percent with a high level of turbidity. A high level of turbidity also increased control by 80 percent in the 3 µg ai L^{-1} fluridone treatments.

Untreated plants at the higher turbidity level also decreased below the initial biomass, with shoot biomass reductions of 42 percent and 72 percent under low and high turbidity levels, respectively (Figure 3A). This result suggests that turbidity alone was suppressing biomass production. Higher levels of turbidity may limit light penetration, restrict photosynthesis, and curtail plant growth (Barko et al. 1986). Although turbidity was not added until plants were near the water surface (~40 cm), turbidity did not prevent curlyleaf pondweed from reaching the surface and forming a canopy. Stems and leaves underlying the surface canopy, however, most likely received less light in turbid water than clear water. Tobiessen and Snow (1984) found that although curlyleaf pondweed could grow under very low light intensities, stems were thinner, fewer in number, and had longer internodes than plants grown under higher light intensities. Biomass, therefore, would be lower in plants grown in lower versus higher light intensities, or turbid versus clear water.

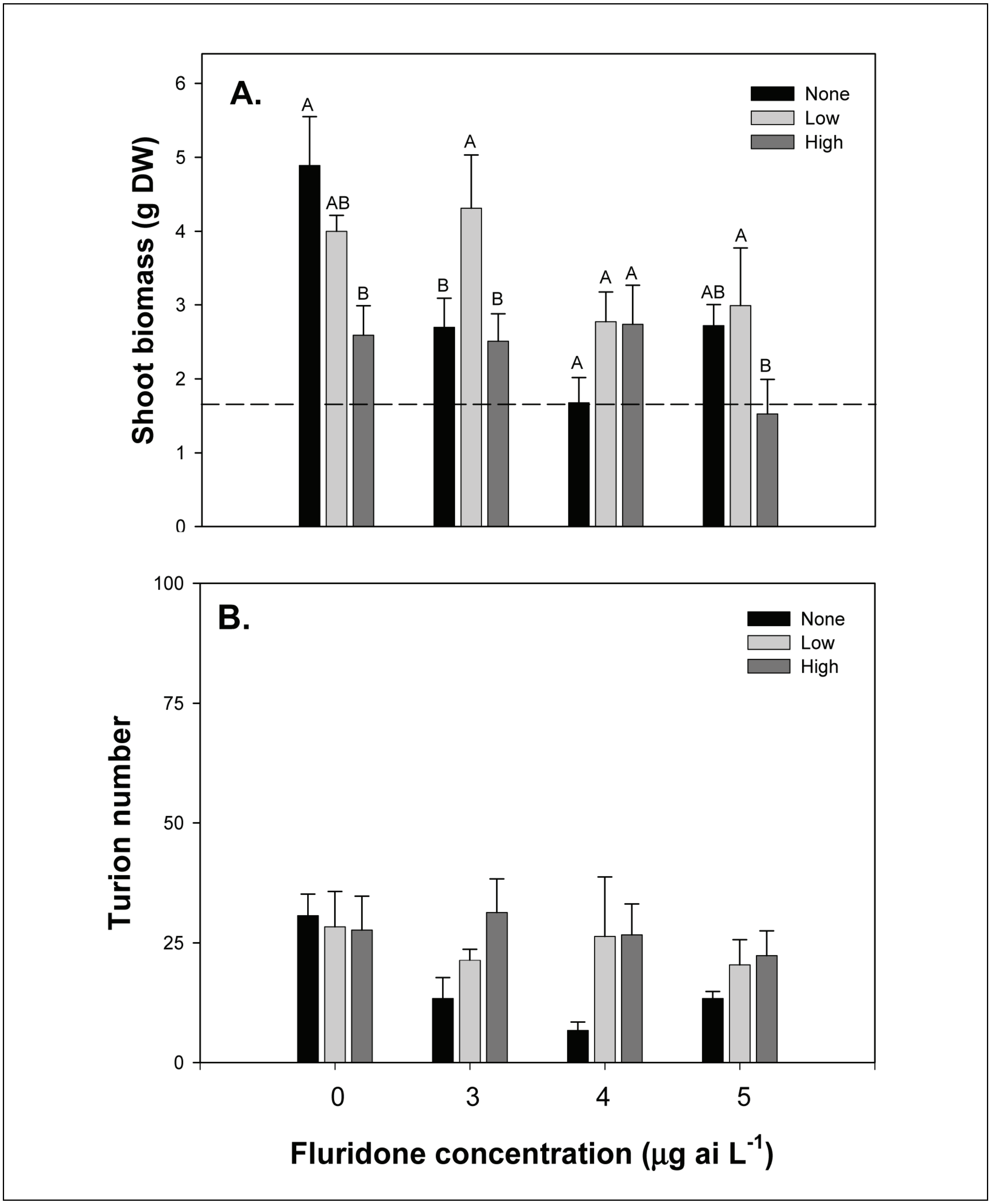

Figure 2. A) Shoot biomass (g DW) and B) total number of turions produced by curlyleaf pondweed 35 days after treatment with 0, 3, 4, and 5 µg ai L^{-1} fluridone under turbid conditions (none, low, and high). Dashed horizontal line indicates biomass at the time of application. Means are ±1 SE (n=3). Different letters denote significant differences between turbidity levels for each fluridone concentration (Tukey p≤0.05).

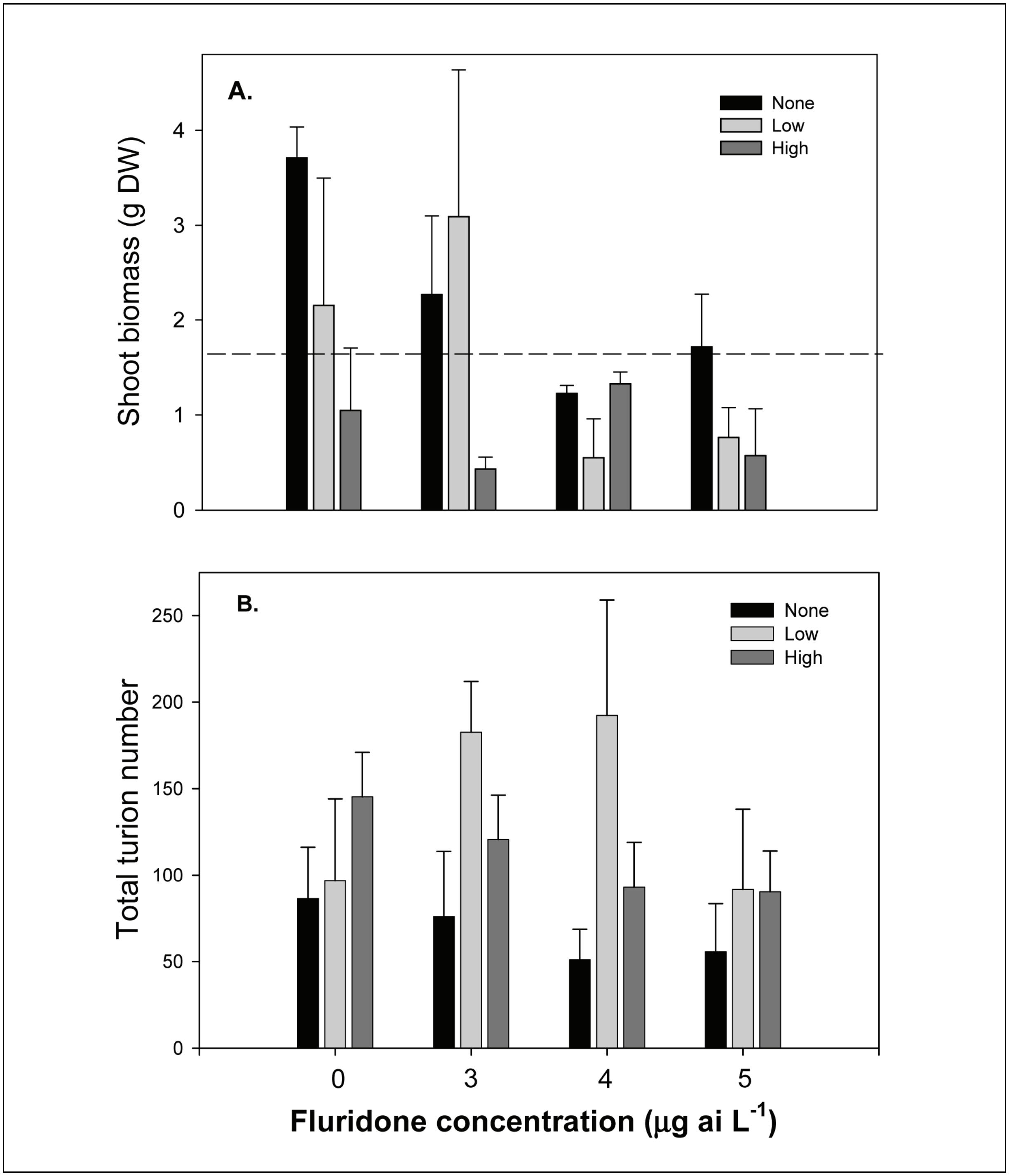

Figure 3. A) Shoot biomass (g DW) and B) total number of turions produced by curlyleaf pondweed 56 days after treatment with 0, 3, 4, and 5 µg ai L^{-1} fluridone under turbid conditions (none, low, and high). Dashed horizontal line indicates biomass at the time of application. Means are ±1 SE (*n*=3). There were no significant differences between turbidity levels for each fluridone concentration (ANOVA, p≤0.05)

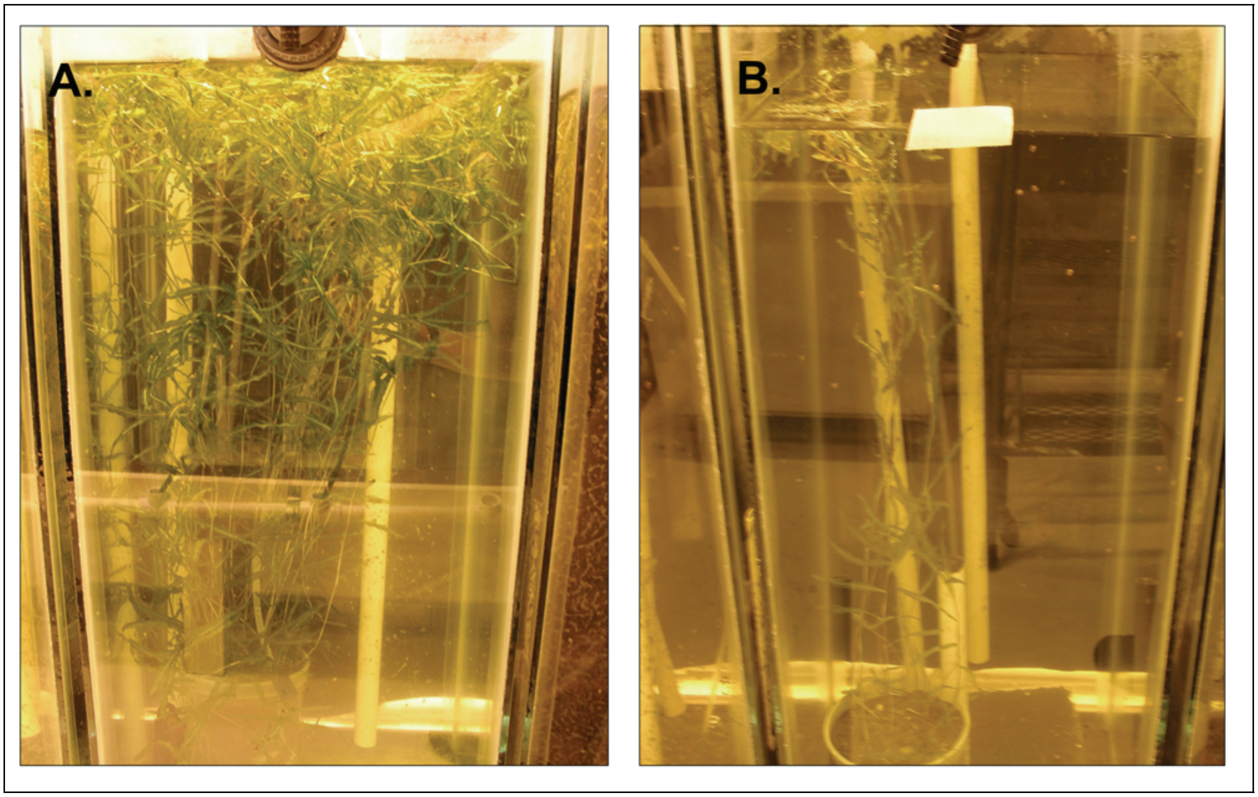

Figure 4. A) Reference aquarium containing untreated curlyleaf pondweed at 56 DAT, and B) treatment aquarium containing plants that were dosed with 3 µg ai L^{-1} fluridone under high turbid conditions at 56 DAT.

Furthermore, plants with fewer leaves and thinner stems would be more susceptible to leaf abrasions and stem damage caused by suspended sediment (Engel and Nichols 1994), which may have contributed to shoot decay and canopy collapse despite the rapid settling of Lake Benton sediment. Plants did not recover when turbidity levels decreased after 21 days, and it is unclear if turbidity levels maintained for less than 21 days would substantially affect plant growth or herbicide efficacy.

Turion Production. At 35 DAT, turion numbers for the reference were relatively similar across turbidity levels, however, a slight trend of increasing turions with turbidity is evident in the fluridone treatments (Figure 2B). This trend was more pronounced by 56 DAT (Figure 3B). Without turbidity, fluridone doses of 3 to 5 µg ai L^{-1} reduced turion numbers by only 12 to 36 percent at the final harvest; however, turion numbers increased in these treatments when turbidity was added (Figure 3B). Plants dosed with fluridone produced two- to fourfold more turions, including numbers of both mature and immature axillary turions, under turbid conditions. Although turbidity was a statistically significant variable for turion numbers at 35 DAT ($F=3.446$, $p=0.048$) and 56 DAT ($F=4.222$, $p=0.027$), there was not a significant interaction between turbidity and fluridone application (35 DAT: $F=0.950$, $p=0.479$; 56 DAT: $F= 1.033$, $p=0.429$); however, trends are apparent at both harvest dates.

Turion production also increased for plants in turbid conditions that were not treated with fluridone (Figure 3B). In culture experiments, van Wijk et al. (1988) reported increased tuber numbers under reduced light intensities for sago pondweed, and speculated that enhanced tuber production could be a physiological adaptation to stress due to unfavorable light conditions (i.e. turbidity). The same might be true for curlyleaf pondweed; however, why turbidity in combination with fluridone exposure decreased biomass, but not turion production, is inconclusive. Biomass decreases concomitant with reduced turion numbers have been reported for other studies in which curlyleaf pondweed was controlled with fluridone (Poovey et al. 2005, 2006), diquat, and endothall (Netherland et al. 2000, Poovey and Getsinger 2002).

Numbers of turions produced by all treatments were comparable to turions found in curlyleaf pondweed stands in southern Minnesota lakes (Woolf and Madsen 2003). Here, plants grew quickly and were near the water surface when dosed with herbicide. Because curlyleaf pondweed turion formation is influenced by temperature and light conditions, it is probable that the higher temperatures and longer day lengths used in this study cued turion formation before herbicide application or early in the exposure period. Sastroutomo (1980) found that turions can be induced at water temperatures ranging from 13 to 24°C and a photoperiod of 12:12. A photoperiod of 14:10 was used in this study and mean water temperatures were 22.4 ±0.01 °C.

Although sediment turion numbers before and after one fluridone application were relatively similar in Lake Benton, turion numbers were reduced by ~50 percent after two consecutive applications of ~4.0 µg ai L^{-1} fluridone. Both frequency and biomass of curlyleaf pondweed was also greatly reduced in the lake.[1] In these field applications, fluridone was applied soon after ice-out (April 11-14) when plants were immature and before they had reached the water surface. Although the water column in Lake Benton is turbid during this time of year, fluridone efficacy apparently has not been limited by turbidity and suspended sediment, which corresponds to the results of this study. The results of this study also indicate that turbidity may augment fluridone efficacy with greater reductions in the curlyleaf pondweed biomass in a turbid versus clear water column; nonetheless, the proliferation of turions in turbid treatments requires further investigation.

SUMMARY: This study was a first step in documenting inorganic turbidity impacts on fluridone efficacy when controlling the invasive species curlyleaf pondweed. Turbidity in the water column did not substantially impact aqueous fluridone residues. Without turbidity, fluridone concentrations of 3 to 5 µg ai L^{-1} for a 56-day exposure period suppressed growth of curlyleaf pondweed by 42 to 72 percent, but only slightly reduced turion production. The addition of turbidity to the water column further reduced shoot biomass for curlyleaf pondweed in all treatments, including the references, by as much as 80 percent; however, turion numbers increased with increased turbidity. Plant maturity at the time of herbicide application probably influenced turion production in this study, as plants may have been already cued to form turions.

FUTURE WORK: Further investigation of the role of inorganic turbidity on plant growth and turion formation would help refine the impacts of environmental factors on fluridone efficacy when controlling curlyleaf pondweed.

[1] Unpublished data, 2007, W. Crowell, Invasive Species Coordinator, Minnesota Dept. of natural Resources, St. Paul, MN.

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